THE *GLN1* LOCUS OF *SACCHAROMYCES CEREVISIAE* ENCODES GLUTAMINE SYNTHETASE

AARON P. MITCHELL[1]

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT

Among 41 yeast glutamine auxotrophs, complementation analysis defined a single gene, *GLN1*, on chromosome *16* between *MAK3* and *MAK6*. Half of the alleles fell into two intragenic complementation classes. No clustering of complementing alleles was found in a fine structure map. Altered glutamine synthetase subunits, including nonsense fragments and charge variants, were identified in several of the mutants, indicating that *GLN1* is the structural gene for this enzyme. Negative complementation was observed for almost every allele associated with a protein product and all *gln1*/+ heterozygotes displayed reduced susceptibility to ammonia repression of the remaining glutamine synthetase activity. This latter observation is explained by the hypothesis that ammonia represses the enzyme only through its metabolism to glutamine. A basis for the two *gln1* complementation classes is proposed.

GLUTAMINE synthetase (EC 6.3.1.2) catalyzes the condensation of ammonia with glutamate to yield glutamine at the expense of an adenosine 5′-triphosphate (ATP) phosphodiester bond. This is among the few ubiquitous biological reactions in which ammonia is used as a substrate and, as such, represents a key step in the assimilation of inorganic nitrogen.

The *Saccharomyces cerevisiae* glutamine synthetase is an oligomer of ten or twelve M_r 43,000 subunits (MITCHELL and MAGASANIK 1983). The level of enzyme activity is regulated by the rate of subunit synthesis and by glutamine-stimulated inactivation (LEGRAIN *et al.* 1982; MITCHELL and MAGASANIK 1983, 1984a). As yet, the structural basis of inactivation is unknown, but it is reversible *in vivo* and does not involve dissociation of the enzyme oligomer.

Synthesis of the enzyme is regulated by three genetically and physiologically distinct systems: glutamine (*GLN3*) control, general amino acid control and purine control (MITCHELL and MAGASANIK 1984b,c). The *GLN3* system is responsible for derepression of several gene products in response to limitation for glutamine; glutamine synthetase depends on this system to achieve its fully derepressed level. General amino acid control (reviewed in JONES and FINK 1982) and purine control promote elevated expression of glutamine synthetase upon starvation for single amino acids and purines, respectively. The enzyme

[1] Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

reaches only one-tenth of its maximal level when either of these latter systems is stimulated.

An understanding of the mechanisms through which these systems act will depend on identifying the basic components of the regulatory networks. This communication provides evidence that the *GLN1* locus is the structural gene for the glutamine synthetase subunit.

MATERIALS AND METHODS

Strains and genetic markers: The strains used in this work are listed in Table 1. Those listed in the upper section are derived from Σ1278b of J. M. WIAME. The *his-4-42, lys-23, ade2-102*, and *met13-25* (amber) mutations were isolated by MARJORIE BRANDRISS; *ilv1-1* (amber), *lys2-1* (ochre), and *leu2-1* (ochre) were crossed into this background five times from SL183-21C. All Gln^- mutations were maintained within this genetic background.

Media: Synthetic media contained, per liter, 3.4 g of Difco yeast nitrogen base without amino acids or ammonium sulfate, 20 g of D-glucose and, as nitrogen sources, either 2.0 g of glutamine (Ggln medium), 2.0 g of ammonium sulfate (GN) or 1.28 g of sodium glutamate (Gglt). Auxotrophic supplements were included when necessary at the concentrations specified by SHERMAN, FINK and LAWRENCE (1978). Both YPD (SHERMAN, FINK and LAWRENCE 1978) and MB (SOMERS and BEVAN 1969) were supplemented with 3 g of glutamine per liter. Glutamine was kept as a filter-sterilized, 3% stock at room temperature for up to 1 wk. Media were solidified by including 20 g of agar per liter.

Genetic methods: Standard procedures were employed in matings, sporulation, ascus dissection and random spore analysis (SHERMAN, FINK and LAWRENCE 1978). The Kil^- phenotype of *mak3-1* and *mak6-1* was scored according to SOMERS and BEVAN (1969). Mutagenesis with ethyl methanesulfonate followed the procedure of CARLSON, OSMOND and BOTSTEIN (1981). Essentially the same protocol was used for mutagenesis with 2.5% 1,2,7,8-diepoxyoctane, except that the treatment was terminated by filtering the cells out of the mutagen. Gln^- mutants were identified by their ability to grow on Ggln, but not on GN, Gglt or on media containing both ammonia and glutamate.

Sunlamp-induced heteroallelic recombination: Gln^+ mitotic recombinants were selected from heteroallelic diploids after sunlamp irradiation (LAWRENCE and CHRISTENSEN 1974) on GN plates supplemented with adenine, uracil and 0.001% glutamine. Recombination rates were calculated from the frequencies of recombinants after four different sunlamp doses. Rough locations for the alleles were determined in crosses with *gln1-3, -5, -10* and *-28*, then neighbors and next-nearest neighbors were identified by comparing appropriate pairwise recombination rates. *gln1-24* gave reduced recombination rates with several alleles and, therefore, was fitted into the previously established linear array; it was inferred to be a single site mutation because it was revertible.

Miscellaneous: Details of culture conditions, preparation of crude extracts, enzyme assays, sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, fluorography and immunoprecipitation of denatured glutamine synthetase subunits have been described (MITCHELL and MAGASANIK 1983; 1984a), except that bovine serum albumin and competitor crude extract were eliminated from the immunoprecipitation reactions and that protein A-Sepharose (Pharmacia) was often used instead of fixed *Staphylococcus aureus* cells to collect immune complexes. Two-dimensional polyacrylamide gel electrophoresis (MILLER, XUONG and GEIDUSCHEK 1982) and immunological blotting (TOWBIN, STAEHELIN and GORDON 1979) followed standard procedures, with only minor modifications (MITCHELL 1984). Cells were pulse-labeled with L-[^{35}S]methionine for 5 min under repressing conditions (10 min after a shift from Ggln to fresh Ggln) or under derepressing conditions (10 min after a shift from Ggln to fresh Gglt), as described previously (MITCHELL and MAGASANIK 1983).

RESULTS

Isolation and basic characterization of Gln^- mutants: Forty-one yeast glutamine auxotrophs were isolated by screening mutagenized populations in four sepa-

TABLE 1

Strain list

Strain	Genotype	Source
(Σ1278b derivatives)		
MB1000	*α*	M. BRANDRISS
269-5D	*α ade2-102 lys-23*	Present work
269-6B	**a** *his4-42 lys-23*	Present work
318-4D	*α met13-25*	Present work
645-2C	**a** *leu2-1 lys2-1 ilv1-1 met13-25*	Present work
Ura⁻ allelism testers		
X1266-1C	**a** *ura1 met10 his4 leu2 ade2 ade4 gal2*	YGSC[a]
368-2D	**a** *ura2-9,15,30 lys-23*	Present work
STX70-1B	**a** *ura3 met2 leu1 gal2*	YGSC
3971-5B	**a** *ura3 ura4 leu2 lys1 aro1D ilv1 met8 ade5,7 gal10 can1 mal SUC*	YGSC
Miscellaneous		
DBY1178	*α ura2-9,15,30*	D. BOTSTEIN
SL183-21C	*α leu2-1 lys2-1 his5-2 ade3-16 met8-1 ilv1-1 trp1-1 aro7-1*	M. BRANDRISS
RW1633	*α arg4 mak3-1 aro7*	R. WICKNER
RW923	*α his2 ade1 leu1 mak6-1 aro7*	R. WICKNER
426	$\frac{\mathbf{a}\ leu2\text{-}1\ +}{\alpha\ +\ arg4} \quad \frac{gln1\text{-}37\ +\ +}{+\ mak3\text{-}1\ aro7}$ [KIL⁺]	Present work
455	$\frac{\mathbf{a}\ his2\ leu1\ ade1}{\alpha\ +\ +\ +} \quad \frac{+\ gln1\text{-}37\ +}{mak6\text{-}1\ +\ aro7}$ [KIL⁺]	Present work
456	$\frac{\mathbf{a}\ +\ leu1\ ade1}{\alpha\ his2\ +\ +} \quad \frac{gln1\text{-}37\ +}{+\ aro7}$ [KIL⁺]	Present work

[a] Yeast Genetic Stock Center.

TABLE 2

Glutamine auxotroph isolations

Isolation	Parent strain	Mutagen[a]	Survival (%)	No. of mutants	Mutation nos.	Total no. screened
A	269-6B	EMS	60	3 Gln^-	1, 2, 37	3×10^4
B	MB1000	DEO	25	1 Gln^-	3	4×10^4
C	318-4D	EMS	19	21 Gln^-	4–24	3×10^4
D	269-5D	EMS	14	16 Gln^-	25–36, 38–41	7×10^4
				14 *ura1*		
				23 *ura2*		
				17 *ura3*		
				19 *ura4*		

Mutagenized populations were spread on plates containing glutamine and the minimal supplements for the parent strain (and uracil, for isolation D). Mutants that failed to grow on glutamine-free replicas were purified and retested; mutants failing to grow on uracil-free replicas from isolation D were placed into complementation groups by crosses with the testers listed in Table 1. Isolation A was carried out at 37°, the others at 30°.

[a] Abbreviations used are: EMS, ethyl methanesulfonate; DEO, diepoxyoctane.

rate experiments, as summarized in Table 2. In the fourth screen (isolation D), Ura$^-$ and Gln$^-$ mutants were isolated simultaneously, then sorted by auxotrophy and by ability to complement Ura$^-$ allelism testers. Gln$^-$ mutations were recovered at the same frequency as members of each of the characterized *URA* complementation groups, which are single, conditionally dispensable genes (JONES and FINK 1982).

In outcrosses of 29 of the mutants, the Gln$^-$ phenotype segregated as a single gene trait. Mutants 1 and 35 exhibited a heat-sensitive glutamine auxotrophy, mutant 22 was a cold sensitive glutamine auxotroph, and mutants 7, 15, and 33 displayed a leaky Gln$^-$ phenotype at all temperatures. Of the mutations recovered from isolations A, B and C, mutations 5, 8, 10, 13 and 37 showed frequent coreversion with the amber allele *met13-25* and were classified as amber suppressible. The remaining mutations from these isolations were found to be revertible.

All of the Gln$^-$ mutations were recessive and failed to complement the amber mutations 5 and 10. The mutation with the leakiest phenotype, 7, yielded only parental ditypes with mutation 5 in 15 tetrads. Therefore, these mutations are alleles of a single gene, which I designate *GLN1*. Isolation numbers will be used as allele numbers below.

Map location of GLN1: Linkage of *GLN1* to the chromosome 16 locus *ARO7* was detected in routine crosses. The map order *CEN16-MAK6-GLN1-MAK3-ARO7* was established by tetrad analysis of diploids 426, 455 and 456 (Table 3), and by comparison with previous mapping data (MORTIMER and SCHILD 1980).

Alleic complementation: Diploids were constructed that carried all possible heteroallelic combinations of the *gln1* mutations from isolations A, B and C, except for *gln1-2*, and complementation was assessed by growth in the absence of glutamine. Three general complementation patterns were displayed, defining three classes of *gln1* alleles (Table 4). The nine class I alleles failed to complement all other alleles, whereas class II alleles (*gln1-1, -7, -19* and *-22*) complemented many of the remaining 11 alleles that constitute class III. There was no complementation between alleles in the same class.

gln1-2 and the mutations found in isolation D were placed in complementation classes by crosses with several class I, II and III testers (Table 4). Ten mutants failed to complement all testers, and seven complemented some or all of the class II testers, placing them in classes I and III, respectively.

Evidence that complementation is interallelic, rather than intergenic, includes data showing (1) that all amber alleles are noncomplementing, (2) that complementation is often weak or heat sensitive and (3) the biochemical characterization of *gln1* protein products (see below).

Fine structure map of GLN1: A map of several *gln1* mutational sites, shown in Figure 1, was assembled from sunlamp-induced heteroallelic recombination rates (Table 5).

The five class I alleles (unenclosed numbers) represent four sites and cover most of the mapped interval; this was expected from their genetic and biochemical properties (see below). Among class III alleles (boxed), some with

TABLE 3

Segregation data for chromosome 16 markers

Interval	Cross	Tetrad type				Total	Recombination (%)
		P	N	T	D		
ARO7-GLN1	426	77	0	45		122	18
	455	47	0	11		58	10
	456	31	0	16		47	17
	pooled	155	0	72		227	16
MAK3-GLN1	426	85	0	36		121	15
MAK3-ARO7	426	115	0	9		124	4
MAK6-GLN1	455	28	1	24		53	28
MAK6-ARO7	455	24	1	30		55	33
CEN16-MAK6	455[a]			10	44	54	8
CEN16-GLN1	455[a]			34	23	57	30

Recombination rates were calculated from numbers of parental ditype (P), nonparental ditype (N), tetratype (T), and ditype (D) tetrads, according to PERKINS (1949).

[a] The centromere marker *LEU1* was used in these determinations, and recombination rates were corrected for its second division segregation frequency of 3% (MORTIMER and SCHILD 1980).

TABLE 4

Complementation properties of gln1 *alleles*

Class	Representative Allele[a]	Class I	Class II				Class III							
		5[b]	*1*	*19*	*22*	*7*	*17* *24*	*15* *21* *23*	*9* *11* *14* *20*	*4* *16*	*2*	*27* *34* *38* *41*	*33*	*39*
I	*5*	–	–	–	–[c]	–	–	–	–	–	–	–	–	–
II	*1*		–	–	–[c]	–	+/–	–	+,+/–	+/–	+/–	+/–	–	–
	19			–	–	–	–	+	+	h	+	+	+	h
	22				–	–	–	–	+	+	+	+	+	+
	7					–	–	–	+,+/–	h	h	+	+	h
III	*17*						–	–	–	–	–	–	–	–
	21							–	–	–	–	–	–	–
	9								–	–	–	–	–	–
	4									–	–	–	–	–

Complementation was assessed by the ability of doubly heterozygous diploids to grow on minimal GN and Gglt media. +, strong growth; +/–, weak growth; h, growth at 23° but not at 37°; –, no growth. The symbol +,+/– indicates that complementation varied from strong to weak with different alleles listed in the same column.

[a] The complementation testers crossed to mutant 2 and the mutants from isolation D carried the class I alleles 5, 10 and 12, the class II alleles *1, 19, 22* and *7* and the class III alleles *17, 24, 21, 23, 9, 11, 4* and *16*. Only one representative with each specific complementation pattern is listed in this column.

[b] Class I includes the alleles *3, 5, 6, 8, 10, 12, 13, 18, 25, 26, 28, 29, 30, 31, 32, 35, 36, 37* and *40*.

[c] Alleles *1* and *35* cause a heat sensitive Gln^- phenotype; allele *22* causes a cold-sensitive Gln^- phenotype. The *gln1-22/gln1-1* and *gln1-22/gln1-35* diploids both display a cold-sensitive glutamine auxotrophy; therefore, these alleles fail to complement.

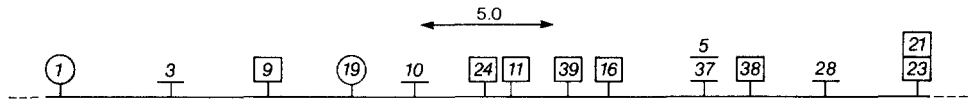

FIGURE 1.—Sunlamp-induced recombination map of the *GLN1* locus. Class I alleles are unenclosed, class II alleles are circled and class III alleles are boxed. The double arrow indicates the distance corresponding to five Gln^+ recombinants per 10^6 cells per min of sunlamp irradiation.

quite similar complementation properties map at distinct sites; for example, mutations *9, 11* and *38* or *16* and *39*. However, six of the seven class III sites are within the right half of the map. The two class II alleles (circled) that were tight enough to map lie within the left half of the interval, but too few class II alleles were mapped to see if the locus has a bipartite structure.

Functional analysis of the GLN1 *gene*: Strains carrying various different *gln1* alleles produced no detectable glutamine synthetase activity during growth under derepressing conditions. The synthetase assay measures γ-glutamyl phosphate produced from glutamate and ATP; synthesis of glutamine would result from subsequent replacement of the phosphate by ammonia. Glutamine synthetases also catalyze a partial reverse reaction: condensation of glutamine and arsenate to form γ-glutamyl arsenate. A structurally altered enzyme could retain this latter "transferase" activity despite its inability to synthesize glutamine *in vivo*. Indeed, *gln1-1, -7* and *-22* mutant extracts possessed transferase activity. Strains carrying *gln1-1* exhibit a heat-sensitive glutamine auxotrophy and heat-sensitive transferase activity in extracts (data not shown), suggesting that *GLN1* is the structural gene for glutamine synthetase. This hypothesis was examined through immunological studies of *gln1* mutant glutamine synthetase subunits.

Extracts of strains carrying amber *gln1* alleles, pulse-labeled with [^{35}S]methionine shortly after derepression, were immunoprecipitated rabbit anti-glutamine synthetase antiserum. Fluorograms of the immunoprecipitates, fractionated by SDS polyacrylamide gel electrophoresis, are shown in Figure 2. The wild-type glutamine synthetase subunit was identified by comparison of repressed and derepressed *GLN1* samples (lanes 6 and 7), in which only a M_r 43,000 immunoprecipitated polypeptide was glutamine repressible. This polypeptide was not detectable in extracts of strains carrying the amber alleles *gln1-5* (lane 3), *gln1-10* (lane 4), *gln1-13* (lane 8), *gln1-8* (data not shown) and *gln1-37* (MITCHELL and MAGASANIK 1983).

Among other class I lesions, *gln1-3, -12* and *-28* also blocked synthesis of full-length glutamine synthetase subunits (Figure 2, lanes 5, 9 and 2), whereas *gln1-18* did not (lane 10). Eight additional class I mutant extracts were fractionated on SDS polyacrylamide gels, and accumulation of full-length subunits was detected by immunological blotting. Alleles *25, 26, 29, 31* and *32* blocked accumulation of the subunit, but *30, 36* and *40* did not (MITCHELL 1984). Thus, the majority of noncomplementing alleles, including all identified amber alleles, produce no full-length glutamine synthetase monomer.

The opposite correlation exists among complementing alleles: seven different class II and III alleles were associated with production of M_r 43,000 subunits,

TABLE 5

Sunlamp-induced recombination rates between Gln⁻ mutations

Gln⁻ mutation in parent 2	Gln⁻ mutation in parent 1														
	1	3	9	19	10	24	11	39	16	5	37	38	28	21	23
1	(0.7)	4.2	L	15	25	L	L	L	L	45	ND	L	43	L	L
3		(0.4)	3.7	7.9	9.9	5.2	11	ND	12	20	14	ND	15	17	19
9			(0.0)	L	3.8	11	ND	ND	27	30	ND	ND	ND	ND	ND
19				(0.6)	2.4	3.5	L	L	L	13	ND	L	16	L	L
10					(0.8)	2.7	7.2	13	21	15	12	20	17	17	40
24						(0.0)	1.0	1.8	2.1	7.0	ND	9.7	24	15	20
11							(0.1)	2.2	3.6	15	ND	ND	ND	ND	ND
39								(0.0)	1.5	4.4	ND	ND	12	ND	16
16									(1.0)	4.3	3.0	3.5	12	14	22
5										(0.0)	0.0	1.0	6.4	9.3	6
37											(0.0)	1.7	ND	ND	ND
38												(0.0)	3.0	7.0	10
28													(0.0)	3.1	3.7
21														(0.0)	0.2
23															(0.1)

Diploids resulting from a cross of parent 1 and parent 2 were analyzed for sunlamp-induced recombination rates, as described in MATERIALS AND METHODS. The rates listed are corrected for the sunlamp-induced reversion rates shown in parentheses. ND, not determined; L, diploid exhibited too leaky a Gln⁻ phenotype to be used.

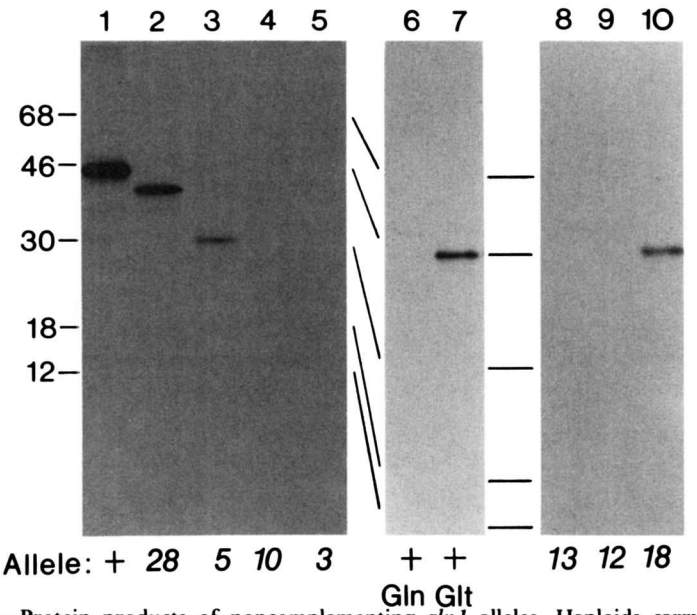

FIGURE 2.—Protein products of noncomplementing *gln1* alleles. Haploids carrying the *gln1* allele indicated under each lane and otherwise of genotype **a** *his4-42 lys-23* (lanes 6–10) or diploids homozygous for each *gln1* allele with otherwise complementing auxotrophies (lanes 1–5) were labeled with [^{35}S]methionine under repressing (lane 6 only) or derepressing conditions. Crude extract proteins bound to rabbit anti-glutamine synthetase antiserum and *S. aureus* protein A were fractionated on SDS polyacrylamide gels and were visualized by fluorography. Extract samples contained either 3×10^6 (lanes 1–5) or 5×10^5 cpm of incorporated radioactivity. The lines at the side of each window indicate the positions of molecular weight standards with sizes in $M_r \times 10^{-3}$.

although occasionally these were recovered by immunoprecipitation in reduced amounts (Figure 3).

That some mutations result in charge changes was revealed by fractionation of *gln1*/+ diploid extracts on two-dimensional gels and immunological blotting (Figure 4). The control *gln1-10*(amber)/+ strain produced a single immunologically reactive spot, whereas diploids heterozygous for *gln1-1, gln1-16* or *gln1-24* produced twin spots. This argues that class II and III mutations cause amino acid substitutions in the glutamine synthetase subunit. It should be noted that alleles at opposite ends of the locus (*1* and *28*; Figure 1) alter the properties of the same polypeptide and, thus, lie in the same coding sequence.

Nature of the gln1-28 *allele*: Strains carrying *gln1-28* produce a M_r 40,000 glutamine synthetase subunit instead of the M_r 43,000 wild-type form. The *gln1-28* site is near one end of the locus, compatible with its being a nonsense allele. However, reversion is infrequent ($<10^{-8}$ in several independent experiments) and usually occurs through back mutations because ten of ten such revertants failed to yield Gln^- meiotic progeny in crosses to a *GLN1* strain.

Nonsense suppressors selected through coreversion of *lys2-1* and *leu2-1* (ochre) or *ilv1-1* and *met13-25* (amber) fail to suppress the *gln1-28* auxotrophy. However, the ochre suppressors were perfectly capable of bypassing the *gln1-*

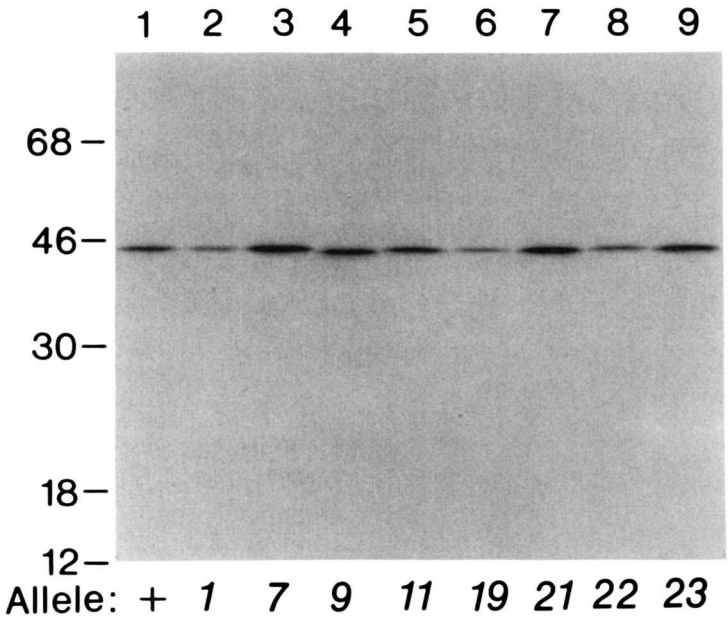

FIGURE 3.—Protein products of complementing *gln1* alleles. Haploids of genotype **a** *his4-42 lys-23* and carrying the *gln1* allele listed under each lane were labeled under derepressing conditions and analyzed, as described in the Figure 2 legend. Extract samples contained 2.5×10^5 cpm of incorporated radioactivity.

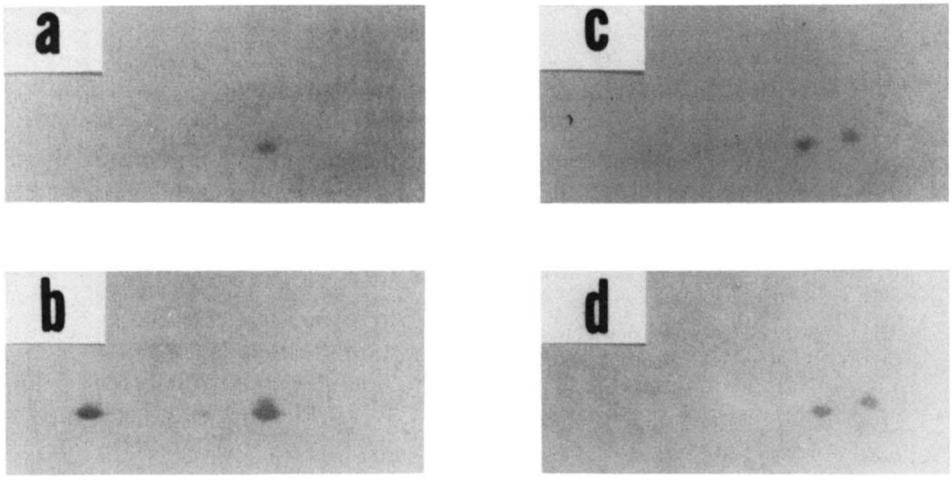

FIGURE 4.—Detection of charge changes associated with *gln1* alleles. Extracts of Gglt-grown cells were fractionated on two-dimensional gels (basic end to the left, SDS gel from top to bottom), and glutamine synthetase subunits were identified by immunological blotting with rabbit anti-glutamine synthetase antiserum. The strains were diploids of genotype +/*gln1-10*, a; +/*gln1-1*, b; +/*gln1-16*, c; and +/*gln1-24*, d.

28 polypeptide termination defect (Figure 5, lanes 3–6); therefore, *gln1-28* is an ochre mutation.

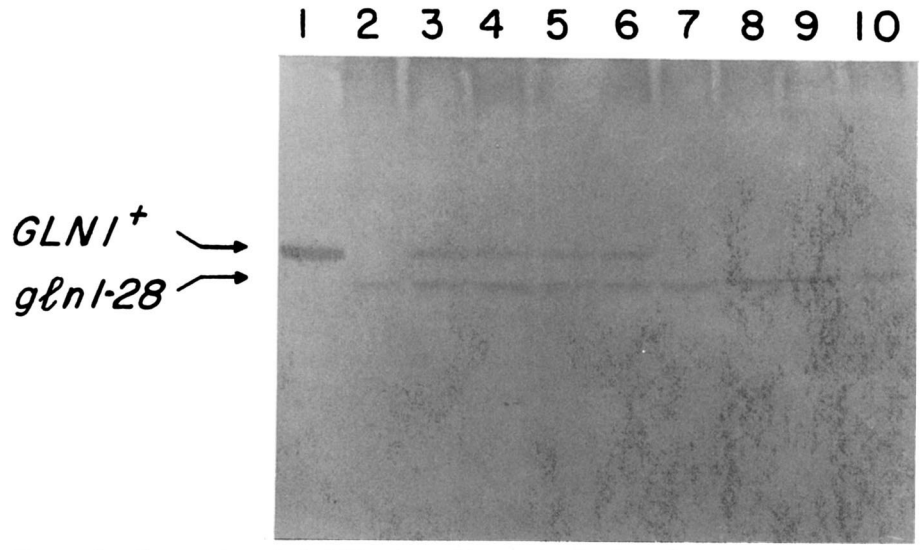

FIGURE 5.—Suppression of *gln1-28* polypeptide termination by ochre suppressors. Strain 695-1B (a *leu2-1 lys2-1 met13-25 ilv1-1 ade2-102 gln1-28*, lane 2), four independent Lys$^+$ Leu$^+$ revertants (carrying ochre suppressors, lanes 3–6), four independent Ilv$^+$ Met$^+$ revertants (carrying amber suppressors, lanes 7–10) and the *GLN1* strain 645-2C (lane 1) were grown on Glt containing 0.01% glutamine and other minimal supplements. Extracts were fractionated on an SDS polyacrylamide gel and were probed with rabbit anti-glutamine synthetase antiserum by immunological blotting.

Dosage studies of gln1 *alleles*: Glutamine synthetase is repressed during growth on Gln medium, derepressed 30- to 40-fold on GN and derepressed approximately 100-fold during growth on Glt (Table 6, wild-type diploids 100 and 100A); no other medium yields higher enzyme levels. The synthetase/transferase ratio is in the range of 0.08 to 0.10.

Diploids heterozygous for one group of class I alleles, designated class IA, maintain a normal synthetase/transferase ratio on Glt. All alleles that produce no material immunologically related to glutamine synthetase are in this group, and the corresponding diploids (103, 108, 110 and 113) produce an average of 60% of the synthetase activity and 53% of the transferase activity found for diploid 100 on this medium.

The remaining *gln1* alleles are associated with aberrant synthetase/transferase ratios in heterozygous diploids, with the exception of *gln1-19*. The thermolability of transferase activity in several such diploids (105, 118, 101, 109 and 116; Table 6) is corroborative evidence that these enzymes are structurally altered. This set of strains displays consistently lower synthetase levels than the class IA heterozygotes on Glt. Immunological evidence demonstrates that at least 14 of these alleles manufacture a protein product (Figures 2, 3 and 4; MITCHELL and MAGASANIK 1983; MITCHELL 1984).

Ten percent of the wild-type synthetase activity is near the minimum required to prevent glutamine limitation, because only strains 101, 130 and

TABLE 6

Dosage effects of gln1 *alleles*

Allele class	Strain	Relevant genotype[a]	Medium: Ggln S	Ggln T	GN S	GN T	GN S/T	Glt S	Glt T	Glt S/T	%T 8 min, 54°
	100	+/+	<0.01	0.02	0.08	0.86	0.09	0.26	3.04	0.08	96
	100A	+/+		0.03	0.09	0.92	0.10	0.28	2.78	0.10	
IA	103	+/*gln1-3*		0.02	0.08	0.72	0.11	0.15	1.49	0.10	95
	106	+/*gln1-6*	<0.01	0.01	0.07	0.69	0.11	0.13	1.50	0.09	
	108	+/*gln1-8*	<0.01	<0.01	0.07	0.70	0.11	0.15	1.63	0.09	
	110	+/*gln1-10*	<0.01	<0.01	0.08	0.78	0.10	0.16	1.68	0.10	
	113	+/*gln1-13*						0.16	1.67	0.10	
	135	+/*gln1-35*						0.14	1.43	0.10	
IB	105	+/*gln1-5*						0.07	1.71	0.04	22
	118	+/*gln1-18*						0.09	1.71	0.05	6
	128	+/*gln1-28*		0.01	0.04	1.06	0.03	0.03	1.00	0.03	
	130[b]	+/*gln1-30*		0.02	0.03	4.20	0.01	0.03	3.93	0.01	
	136[b]	+/*gln1-36*						0.10	1.79	0.06	
	137	+/*gln1-37*	<0.01	0.03	0.05	1.41	0.04	0.06	1.86	0.03	
	140[b]	+/*gln1-40*						0.10	1.71	0.06	
II	101	+/*gln1-1*	<0.01	0.02	0.03	2.94	0.01	0.03	3.25	0.01	13
	107	+/*gln1-7*		0.02	0.08	3.99	0.02	0.09	3.52	0.03	
	119	+/*gln1-19*	<0.01	0.02	0.08	0.95	0.09	0.15	1.60	0.09	
	122	+/*gln1-22*	<0.01	<0.01	0.06	1.14	0.06	0.10	1.81	0.06	
	D122	*gln1-22/gln1-22*		<0.01				0.03	0.18	0.20	
III	109	+/*gln1-9*						0.10	1.74	0.06	65
	116	+/*gln1-16*	<0.01	<0.01	0.07	1.25	0.06	0.10	1.71	0.06	6
	123	+/*gln1-23*	<0.01	0.01				0.07	1.38	0.05	
	124	+/*gln1-24*	<0.01	0.02	0.07	1.39	0.05	0.09	1.72	0.05	
	138	+/*gln1-38*						0.08	1.27	0.07	

Cultures were grown at 30° on the media indicated and were harvested during exponential growth. Extracts were assayed for the synthetase (S) and transferase (T) activities of glutamine synthetase and for protein. The enzyme activities are given in units per milligram of protein, and the ratio of synthetase to transferase activity (S/T) is also shown. Extracts of several diploids were adjusted to 2 mg/ml and were heated at 54° for 8 min; the percentage of transferase activity remaining is listed in the %T column.

[a] All strains were of genotype $\frac{a}{\alpha}\ \frac{his4\text{-}42}{+}\ \frac{lys\text{-}23}{+}\ \frac{+}{met13\text{-}25}\ \frac{gln1\text{-}x}{gln1\text{-}y}$ except 100A, 130, 136, and 140, which were $\frac{a}{\alpha}\ \frac{ade2\text{-}102}{+}\ \frac{lys\text{-}23}{+}\ \frac{+}{met13\text{-}25}\ \frac{gln1\text{-}x}{gln1\text{-}y}$

[b] These diploids resulted from crosses to the original Gln$^-$ isolates.

D122 grew more slowly than the wild-type diploids on Glt (220–300 min doubling times *vs.* 140–160 min).

Class IA alleles display a clear dosage effect on Glt, but not on GN. For all classes of alleles, those heterozygotes that produce at least 0.07 units of synthetase per milligram on Glt are repressed to about 0.07 units/mg on GN. Those with less activity on Glt are repressed little, if at all, on GN. This suggests that there is a homeostatic mechanism to maintain a set level of synthetase activity on GN.

DISCUSSION

All characterized single mutations that cause a tight Gln^- phenotype in *S. cerevisiae* are *gln1* alleles, at the structural gene for glutamine synthetase. Every indication is that these mutations include simple loss of function defects. No such straightforward situation exists among Gln^- mutants of other microorganisms. The distinguishing features of the *S. cerevisiae* system are that no glutamine synthetase isozymes apparently exist as they do in Rhizobium (DARROW and KNOTTS 1977; LUDWIG 1980) and in *Neurospora crassa* (DAVILA *et al.* 1983) and that any one of three independent regulatory systems can derepress the enzyme (MITCHELL and MAGASANIK 1984c), in contrast to the case in enteric bacteria (MAGASANIK 1982) and, tentatively, in *Bacillus subtilis* (REYSSET 1981).

It is tempting to speculate that the two classes of complementing *gln1* alleles reflect two general ways to block enzymatic function. For example, such mutations might cause either denaturation of the catalytic center or a mimicking of the conformation associated with glutamine-stimulated inactivation. Mutations with the latter property should be rare because a specific structural change is demanded; class II is the rare class. Indeed, *gln1-1* and *gln1-7* extracts display a very low synthetase/transferase ratio (A. P. MITCHELL, unpublished observations) similar to that of the inactivated wild-type glutamine synthetase (LEGRAIN *et al.* 1982; MITCHELL and MAGASANIK 1984a). Moreover, intragenic suppression of *gln1-1* can produce an inactivation-resistant phenotype (A. P. MITCHELL, unpublished observations). But direct evidence bearing on this model will, no doubt, require a better biochemical understanding of glutamine synthetase and of the inactivation process.

One peculiar characteristic of *gln1* mutations is that virtually every allele associated with a protein product displays negative complementation in *gln1/+* diploids. This is particularly striking in the case of *gln1-22* because the homozygous diploid D122 exhibits significant synthetase activity (Table 6). In addition, the *gln1-5* (and *-37*) and *gln1-28* nonsense fragments are extremely potent inhibitors: heterozygous *gln1/+* diploids manufacture eightfold more wild-type subunits than nonsense fragments (A. P. MITCHELL, unpublished observations). Thus, about one fragment per glutamine synthetase oligomer (of ten or 12 subunits) can inhibit over one-half of its activity.

The *gln1-28* allele is unusual in that ochre suppressors can bypass its polypeptide termination defect, but do not restore glutamine synthetase activity. This may simply indicate that the amino acid required at the *gln1-28* site

cannot be inserted by ochre suppressor alleles of nonessential tRNA genes. However, the *gln1-28*/+ diploid has such severely reduced synthetase activity (Table 6), with an eightfold excess of wild-type subunits over ochre fragments, that even insertion of the wild-type amino acid at the *gln1-28* site by an ochre suppressor might not restore enzyme activity, because of the persistence of the inhibitory ochre fragment (Figure 5).

All *gln1*/+ diploids exhibit impaired ammonia repression of glutamine synthetase. This simply contradicts the proposal that ammonia directly represses the enzyme (LEGRAIN *et al.* 1982). It has been demonstrated previously that the protein P200 and NAD-dependent glutamine dehydrogenase activity, both under *GLN3* control, respond to ammonia only if it is metabolized to glutamine (MITCHELL and MAGASANIK 1984b). If ammonia represses glutamine synthetase only through its metabolism to glutamine, then ammonia should not repress the enzyme below that level of activity required to synthesize the appropriate amount of glutamine. Indeed, the observations in Table 6 indicate that ammonia cannot repress synthetase activity to less than 0.05 units/mg.

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